The low dose damage response pathways in the mouse mammary glands depends on genotype, tissue compartment, exposure regimen, and sampling times

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The objectives of this research are to characterize the early and persistent low-dose and adaptive response (AR) damage surveillance networks in mammary glands of radiation sensitive and resistant strains of mice to identify the molecular signatures/mechanisms associated with non-linear modifications of risk for mammary gland cancer. Our approach uses low-dose exposure regimens that have been reported to induce mammary gland cancer in sensitive strains to determine whether low-dose induced pathways are differentially expressed in epithelial or stromal cells and to determine the temporal pattern of expression of the low-dose pathway. We conducted a time-response analysis of gene expression profiles in the mammary gland (MG) and inguinal mammary gland-embedded lymph node (MG-LN) of BALB/c and C57BL/6 mice that were exposed to a regimen of repeated low dose exposures (four weekly exposure to 7.5 cGy) which has been shown to protect mice against radiation-induced thymic lymphoma from higher doses (4 x 1.8 Gy). The fourth MG and MG-LN were removed at 10 hr or 1 month after the last irradiation and processed for transcriptome analyses using Affymetrix microarrays. The distal part of the MG containing a second LN was embedded in OCT and stored for confirmatory immunohistochemestry studies of cell-type specific expression of genes identified by gene expression analysis. We used system biology, biostatistical, and bioinformatics approaches to generate gene lists with significant expression changes (+/- 1.5 fold and 0.05 p-value) and to identify networks and pathways that were differentially affected by radiation and by time in either cell compartment of the mammary glands of these two strains. The microarray findings showed distinctly different gene expression patterns between BALB/c and C57BL/6 mice and a dramatically different response between the MG and MG-LN within both strains. In BALB/c, the early MG response to low dose involved unique cytokines and transcription factors, while the MG-LN response involved predominantly a downregulation of mitotic genes. Bioinfomatics approaches of clustering, Ingenuity and Gene Ontology demonstrated that the low-dose response in the MG of BALB/c mice included upregulation of epithelial differentiation, prolactin signaling and hypoxia, and downregulation of leukocyte extravasation signaling, integrin signaling and immune response. None of these pathways were affected in the LN of BALB/c mice or C57BL/6 MG and MG-LN. Analysis of MG tissue collected 1 month after the last irradiation, showed a substantially different pattern with respect to that observed at the earlier sampling time after exposure pointing to the transient nature of the majority of the low dose expression responses. Interestingly, there was a significant upregulation of mitotic networks in the BALB/c MG and downregulation in the C57BL/6 MG, further confirming the differential response of these two strains to low dose. These findings suggest that the genotype determines the direction of the low-dose responses of the mitotic networks in mammary gland epithelium and stroma of BALB/c and C57BL/6 mice. These results points to a differential immuno- and inflammatory response in the MG of these two strains and suggest that the BALB/c MG stroma may be more sensitive to or not as efficient in repairing radiation-induced tissue damage as the C57BL/6 stroma resulting in a persistent inflammatory response in BALB/c MG. Further studies

are underway to utilize immunohistochemistry to characterize the cell types involved in the differential inflammatory response, epithelial differentiation responses, leukocyte extravasation, and the other differential response pathways after low dose exposures. We are planning new experiments to compare these low dose signatures with those of different low-dose regimens that have also been reported to modulate risks of radiation induced mammary gland cancer in mouse models.

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